When formed under normal conditions from a single diblock copolymer, polymersomes are spherical and show no asymmetry. However, starting from vesicles of fixed sizes (and therefore of fixed surface area) that encapsulate an organic solvent, one can generate asymmetry by removal of the organic solvent from inside the vesicle, through dialysis. This step, first carried out by the same group in 2010 (ref. 9), reduces the internal volume whilst maintaining the same surface area, resulting in the polymersome wall collapsing in on itself from a single point, in turn creating asymmetric (bowl-shaped) polymersomes called stomatocytes.

In the present work, diblocks containing polyethylene oxide (PEO) and polystyrene (PS) — hydrophilic and hydrophobic, respectively — are used to form vesicles in a mixture of tetrahydrofuran (THF), dioxane and water. The inner compartment of the vesicles contains a fraction of these solvents, as does the hydrophobic part (PS) of the polymersome wall. This confers the required flexibility on the hydrophobic domain, but as PS has a high molecular weight the domain becomes glassy on removal of the organic solvents, and 'freezes' in the bowl-shaped stomatocyte structure. The size of the opening in the stomatocyte may also be controlled by altering the ratio of THF to dioxane.

In this study, the researchers have now conducted the shape transformation in the presence of suitably sized platinum nanoparticles, which become physically entrapped within the opening of the stomatocyte, in turn generating a structure analogous to that of platinum-coated Janus-type nano-swimmers[6] demonstrated for micrometre-sized polystyrene colloids. It is worth noting that the platinum nanoparticle is physically trapped in the exterior surface of the polymersome — not encapsulated within the interior — and so is able to react with the bulk solution. When the platinum-loaded polymersomes are placed within a fuel of hydrogen peroxide, asymmetric breakdown of H_2O_2 into water and oxygen occurs. This alters the osmotic pressure near the platinum nanoparticle, and propulsion is achieved. In this instance, propulsion can occur either through bubble release (although any bubbles formed are too small to be observed by microscopy) or through a self-diffusiophoretic effect, where motion would arise from the local concentration gradient of oxygen generated by the catalytic reaction[10].

Tracking polymer assemblies that are only a few hundred nanometres in size is problematic — here, the researchers resorted to a combination of laser illumination and particle tracking. Most of the observed motion is random — but taking into account the fixed small size of the stomatocytes and the fixed viscosity of the solution, and determining the translational diffusion from the trajectories, an increase is observed in the diffusion coefficient measured, which results in the stomatocytes appearing smaller than they should be. This is interpreted as a propulsive effect.

Where this approach truly advances the field of nanoscale autonomous propulsive devices is that these swimmers were constructed through a bottom-up approach, rather than engineered and fabricated using relatively complicated and demanding methods. Here, entrapping metal nanoparticles within the opening of all the stomatocytes simply by removing the organic solvent leads to the ability to make large quantities of these devices, all identical, all propulsive. Carrying an important chemical cargo encapsulated within a 'fleet' of propulsive vesicles therefore becomes a real possibility. ❐

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PROTEIN LABELLING

Playing tag with proteins

Fluorescent labels can now be attached to a specific protein on the surface of live cells using a two-step method that reacts a norbornene — introduced using genetic encoding — with a variety of dyes.

Dante W. Romanini and Virginia W. Cornish

The introduction of green fluorescent protein (GFP) transformed live-cell imaging because it enabled individual proteins to be selectively labelled within the complex cellular milieu with minimal perturbation of the cell. More recently 'chemical tag' technologies have reduced the size of the label and allowed the use of different fluorophores for varying applications in a modular fashion[1], but the ultimate goal of protein imaging remains the selective labelling of a protein with a small, photostable fluorescent dye without adding or altering anything else in that protein's makeup. Writing in *Nature Chemistry*, Jason Chin, Alexander Deiters and co-workers[2] now describe an important step towards that aim (Fig. 1).

The benefit of fluorescent protein tags is their simplicity — they can be used to selectively tag a single gene within the entire genome, they are produced by the cell's own protein synthesis machinery, and very little must be done to the cell beyond introduction of a recombinant gene. With a mass of ~27 kDa, however, the GFP label is as large as many of the proteins it is used to tag, raising concern that it is perturbing the function and interactions of the biological pathway it is meant to study. Furthermore, despite many examples of successful engineering of fluorescent proteins, its basic structure cannot be drastically altered to introduce new properties. Chemical tagging strategies offer an alternative to GFP — a smaller protein or even a short peptide that can selectively recognize and react with a dye-containing small molecule is fused to the protein of interest. Then, in a second step, the fluorescent tag is added. This method offers a much wider range of options for the dye but requires several amino acids to be added to the protein even for the smallest tags. As a potential alternative, recent advances in our ability to introduce

unnatural amino acids (UAAs) into proteins in living cells have raised the possibility that a bright, photostable fluorescent amino acid could be directly incorporated into a growing polypeptide *in vivo*.

Site-specific introduction of UAAs into proteins by the ribosome was first demonstrated in a test tube using chemically modified transfer RNAs (tRNAs) bearing the desired amino acid and an *in vitro* translation system[3]. Extension of this capability to living cells, however, required several new components. In 2001 Schultz and co-workers showed that mutagenesis to introduce UAAs could be achieved in *Escherichia coli* using an evolved tRNA and its associated amino-acylating synthetase enzyme from a different organism[4]. These translational components were orthogonal, meaning that they did not interact with the bacteria's native tRNAs or amino acids. The foreign tRNA recognizes the UAG codon — a trinucleotide that typically signifies the end of a protein. In this way, the UAA is inserted into a growing protein only at an engineered stop codon, leaving the rest of the protein unaltered. Since then, diverse UAAs have been incorporated using this technology, and recent progress[5] has greatly improved our ability to use this method in mammalian cells.

Despite these advances, it has proved difficult to incorporate fluorophores with sufficient brightness and photostability for live-cell imaging. Amino acids containing dansyl, anap and coumarin dyes have been inserted into proteins in simple bacteria or yeast cells, but have yet to be incorporated in mammalian cells. Other dyes, including a nitrobenzoxadiazole and a BODIPY, have been incorporated in *Xenopus* oocytes — a popular model system with large cells — but the microinjection technique used in these studies is not amenable to the vast majority of mammalian cell lines. The current assumption is that large fluorophore molecules are poor substrates for synthetase enzymes, translational elongation factors, the ribosome itself, or some combination of these components.

It has recently been shown[6] that it is possible to chemically attach a norbornene to a protein chain and then use this strained bicyclic alkene to react with a tetrazine containing dye in a Diels–Alder cycloaddition. Building on this, Chin, Deiters and co-workers now describe the introduction of the norbornene in a site-specific fashion by UAA mutagenesis thus allowing these labelling reactions to be performed in living cells (Fig. 1c).

The advantage of the norbornene cycloaddition is that it is considerably faster than many other 'bioorthogonal' chemical labelling reactions. The reported second-order rate constants are on the order of 1–10 M^{-1} s^{-1} — significantly higher than the ~8 × 10^{-2} M^{-1} s^{-1} previously determined as the upper limit for cell-friendly azide–cyclooctyne cycloadditions[7], though more recently reported cycloalkynes exhibit rate constants that approach that of the norbornene system. This emphasis on kinetics raises an important question that must be answered as researchers continue their efforts to replace GFP with chemical labelling methods: have we yet, or will we ever, develop a reaction that is fast enough to be useful for studying dynamic processes in living cells? An interesting point of comparison here is the current state-of-the-art enzyme-based SNAP chemical tag[8] that has second-order rate constants of up to 1 × 10^4 M^{-1} s^{-1}. Although these rates seem to be sufficient in at least some circumstances, no one has yet explicitly determined the minimum rate necessary for useful intracellular protein labelling. Notably, in this report the groups of Chin and Deiters only show labelling of proteins on the surface of mammalian cells, suggesting that the norbornene reaction may not be fast enough for labelling in the cytosol.

Installing the fluorophore in a second chemical step can be advantageous. It can be designed to be fluorogenic — meaning that fluorescence is greatly increased after the labelling reaction and thus background fluorescence from unreacted dye is reduced. The norbornene system is particularly good in this regard, as the tetrazine group also acts as a fluorescence quencher, resulting in a 5–10-fold increase in fluorescence after the reaction. The improved kinetics of this reaction may also reduce background fluorescence by requiring less reactive dye to achieve sufficient labelling.

The details of the norbornene system also highlight a major technical challenge in the field of UAA mutagenesis, that of finding a tRNA/synthetase pair that can faithfully incorporate the desired UAA while remaining orthogonal to endogenous mammalian cellular machinery. Traditional directed evolution approaches to engineering these components cannot be performed in mammalian cells, and thus considerable effort has been devoted to developing ways to evolve proteins in lower organisms such as yeast and then shuttle them to mammalian cells. The norbornene amino acid avoids this difficulty because it can be incorporated using a wild-type tRNA and synthetase (from single-cell archaea) for pyrrolysine — the twenty-second natural amino acid. These pyrrolysine components have become quite popular in the UAA mutagenesis field because of their inherent orthogonality to bacterial and eukaryotic components and their promiscuity for a range of UAAs, and their utility is sure to expand once more engineered variants are produced.

The question of orthogonality extends beyond just acylation of tRNAs; background incorporation of UAAs at UAG codons other than those introduced for labelling purposes is an under-investigated issue in this field. An earlier report has examined the mechanism by which wild-type stop codons are recognized[9], and the groups of Chin and Deiters begin to address this issue with a qualitative experiment showing preferential

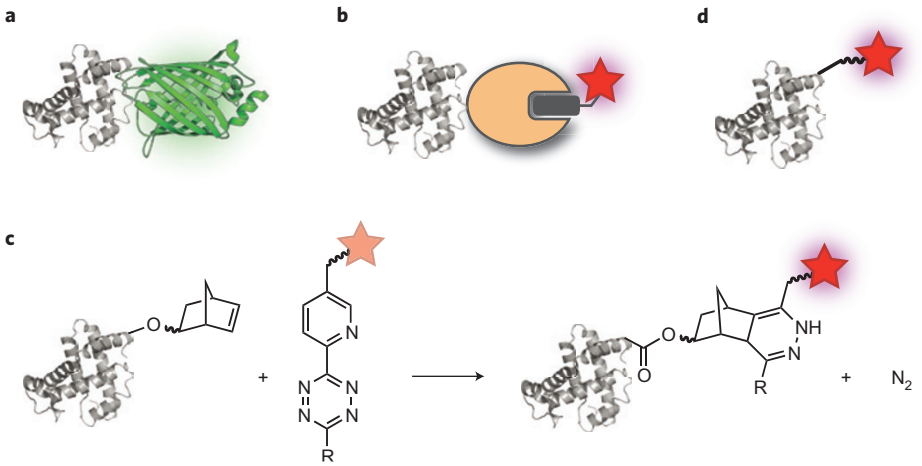

Figure 1 | Strategies for fluorescent tagging of proteins. **a**, Genetic fusions to fluorescent proteins are simple but introduce a large protein domain that could perturb the system being studied. **b**, Chemical tag strategies use smaller protein fusions and enable the use of modular fluorescent dyes with different photophysical properties. **c**, The method developed by Chin and Deiters[2] introduces a reactive norbornene amino acid into the backbone of the protein, which can then react selectively with tetrazines labelling the protein with modular dyes. **d**, Ultimately the direct incorporation of a fluorescent amino acid may be the best way to tag proteins in live cells.

reactivity of their target protein among the entire *E. coli* proteone with a tetrazine dye. However, more detailed studies, including analyses of the effects of such background labelling in mammalian cells, are still lacking.

By combining a new norbornene-containing amino acid and fluorogenic reactive dyes, a modular yet unobtrusive way to label cell surface proteins with photostable organic fluorophores has been developed. Further optimization is likely to be required to achieve labelling within the cytosol and cellular compartments. As it stands, however, this should be a practical tool for biologists, and we look forward to seeing how this technology is applied to study natural phenomena on cell surfaces. ❐

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ZEOLITE NANOSYSTEMS

Imagination has no limits

The technological relevance of zeolites, the desire to improve their efficiency and the inexhaustible synthetic options to tailor their properties have triggered a permanent evolution of this superclass of materials. Two zeolite nanosystems prepared by distinct approaches reflect this and offer hope for new applications.

Javier Pérez-Ramírez

The field of zeolites has rapidly and continually evolved since the introduction of the first synthetic zeolites in the 1950s. A wave of research in the 1980s and 1990s launched the discovery of new framework structures and compositions, serving as adsorbents, ion exchangers and catalysts for the production of fuels and chemicals and for environmental protection. As of today, 204 unique zeolite framework types have been identified[1], although only a handful of them have been commercialized. But not all that glitters is gold. Have you ever experienced the chaos of driving in a large metropolois consisting only of winding and narrow streets, as in Fig. 1a? The charm and possibilities of the city can be spoiled by the heavily congested traffic. A similar situation exists in zeolites, which contain micropores of molecular dimensions (<1 nm). This unique feature, essential for traditional molecular-sieve and shape-selective applications, can be a burden. Bulky molecules face impeded access, getting stuck in pore openings or finding their passage to active centres within the microporous crystal hindered. Such constraints lead to under-use of the available zeolite volume, translating into suboptimal activity, selectivity or lifetime — the key performance indicators of any catalyst. This downside triggered research that targeted improved molecular accessibility and transport by means of wide-pore and/or hierarchically organized zeolites[2].

Hierarchical zeolites aim to combine the intrinsic microporosity — and associated properties such as crystallinity, thermal stability, acidity and reactivity — with an auxiliary network of interconnected meso- or macropores, or both. Rather than the tangled streets of Fig. 1a, the structures are more like the street views shown in Fig. 1b,c. A wealth of new synthetic strategies has recently led to the emergence of a wide range of hierarchically organized zeolitic systems with exciting potential applications[2–5]. Now, two recent studies describe bottom-up approaches to synthesize new zeolite nanosystems of related framework types, specifically ultrasmall crystals of the EMT-type zeolite[6] and hierarchical assemblies of mesoporous faujasite-type (FAU) nanosheets[7].

The group led by Mintova reports in *Science*[6] how control over the very early stages of crystallization in colloidal systems is crucial to isolate important nanosized metastable phases with high purity and yield, while avoiding the use of expensive organic templates. This is illustrated for the EMT-type zeolite, a hexagonal polytype of the cubic faujasite-type (FAU) zeolite, which features a three-dimensional large (12-membered ring) micropore system and one of the lowest zeolite framework densities. The difficulty of obtaining pure EMT, only possible through the use of a costly structure-directing agent (18-crown-6 ether) under tightly controlled synthesis conditions[8], has hampered the wider use of this zeolite. Mintova and co-workers found the trick of 'catching' EMT in template-free solutions: cook the synthesis gel gently for a short time only. High temperatures or long syntheses transform the EMT structure of the first metastable crystalline product into those of the more stable cubic faujasite and of the denser sodalite. One can easily foresee the possibility of capturing other important zeolite phases by deeper inspection of the very early stages of synthesis, thus avoiding the use of organic reagents that are commonly needed to stabilize the desired phases. The achievements in this paper go one step further. Synthesis at near-ambient conditions yields tiny EMT nanocrystals (6–15 nm or 50–70 nm depending on the heating method and dwell time, with a silicon to aluminium ratio of around 1), in contrast to the micrometre-sized EMT crystals obtained by conventional template-assisted crystallization. In this nanosized system (resembling the organization in Fig. 1b) reactant molecules could in principle enter through many pore openings because of the presence of textural mesoporosity, staying in the diminutive microporous domains for a very short time.

Taking a different approach, the team led by Schwieger has engineered a hierarchically organized FAU-type zeolite X, which they report in *Angewandte Chemie International Edition*[7]. This aluminium-rich zeolite, which is a common adsorbent that is industrially synthesized without a template, had previously not been convincingly attained in hierarchical form. Grounded on a soft-templating approach conceived by Ryoo's team for other frameworks[9], this is now a